BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

CLAIMS

1. A process for producing transglutaminase having an enzymatic activity comprising:

(a) incubating a denatured transglutaminase in an acidic aqueous medium; and

(b) adjusting the pH of said aqueous medium to a neutral pH.

2. The process as claimed in claim 1, wherein the aqueous medium further comprises a reducing agent.

3. The process as claimed in claim 2, wherein the reducing agent is selected from the group consisting of dithiothreitol, 2-mercaptoethanol, and tris-(2-carboxyethyl)phosphine.

4. The process as claimed in claim 1, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

5. The process as claimed in claim 4, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

6. The process as claimed in claim 4, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

7. The process as claimed in claim 1, wherein the aqueous medium in step (a) further comprises a protein denaturant.

8. The process as claimed in claim 7, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

9. The process as claimed in claim 7, wherein the transglutaminase concentration is from 40 mg/ml and the protein denaturant concentration is from 4 to 10 M.

10. The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 2 to 7.

11. The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3 to 5.

12. The process as claimed in claim 1, wherein the acidic aqueous medium in step (a) is of a pH from 3.5 to 4.5.

13. The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 5-fold.

14. The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 10-fold.

15. The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted from 50-fold to 400-fold.

16. The process as claimed in claim 1, wherein said incubation is performed at not more than 15°C.

17. The process as claimed in claim 1, wherein said incubation is performed from 3 to 10°C.

18. The process as claimed in claim 1, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted to a concentration of not more than 10 mg/ml.

19. The process as claimed in claim 1, wherein said neutral pH is from 5.8 to 8.5.

20. The process as claimed in claim 1, wherein said neutral pH is from 6 to 7.

21. The process as claimed in claim 1, wherein in step (b), the aqueous medium further comprises an accelerator for forming a higher-order native-state transglutaminase structure having enzymatic activity.

22. The process as claimed in claim 21, wherein the accelerator is selected from the group consisting of an inorganic salt, an organic salt, an amino acid salt, a polyol, an organic solvent, and a surfactant.

23. The process as claimed in claim 21, wherein the inorganic salt accelerator is selected from the group consisting of calcium chloride and strontium chloride.

24. The process as claimed in claim 21, wherein the inorganic salt accelerator concentration is from 0.01 to 10 mM.

25. The process as claimed in claim 21, wherein the organic salt accelerator is selected from the group consisting of sodium acetate and sodium propionate.

26. The process as claimed in claim 21, wherein the organic salt accelerator concentration is from 0.1 to 2 M.

27. The process as claimed in claim 21, wherein the amino acid salt accelerator is arginine hydrochloride.

28. The process as claimed in claim 21, wherein the amino acid salt accelerator concentration is from 0.1 to 2 M.

29. The process as claimed in claim 21, wherein the polyol accelerator is polyethylene glycol.

30. The process as claimed in claim 21, wherein the polyol accelerator concentration is from 1 to 10%.

31. The process as claimed in claim 21, wherein the organic solvent accelerator is selected from the group consisting of DMSO and DMF.

32. The process as claimed in claim 21, wherein the organic solvent accelerator concentration is from 10 to 40%.

33. The process as claimed in claim 21, wherein the surfactant is CHAPS.

34. The process as claimed in claim 21, wherein the surfactant concentration is from 1 to 50 mM.

35. The process as claimed in claim 1, further comprising:

(c) centrifugating the aqueous medium of (b).

36. An isolated transglutaminase obtained by the process of claim 1, which has an intermediate structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

37. The process as claimed in claim 1, wherein step (b) further comprises incubating the aqueous medium for more than 1.5 hours subsequent to adjusting the pH to a neutral region.

38. A process for producing transglutaminase having an enzymatic activity, which comprises subjecting denatured transglutaminase to the following steps (a) and (b):

(a) a step for forming an intermediate structure in which said transglutaminase in the denatured state is incubated in an aqueous medium under acidic conditions; and

(b) a step for forming a higher-order native-state structure exhibiting enzymatic activity by adjusting the pH of the aqueous medium having said intermediate structure to a neutral pH.

39. The process as claimed in claim 38, wherein the aqueous medium further

comprises a reducing agent.

40. The process as claimed in claim 39, wherein the reducing agent is selected from the group consisting of dithiothreitol, 2-mercaptoethanol, and tris-(2-carboxyethyl)phosphine.

41. The process as claimed in claim 38, wherein the denatured transglutaminase is obtained by a process comprising denaturing transglutaminase, which is expressed in a recombinant host cell, in the presence of a protein denaturant.

42. The process as claimed in claim 41, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

43. The process as claimed in claim 41, wherein the transglutaminase concentration is from 10 to 100 mg/ml and the protein denaturant concentration is from 4 to 10 M.

44. The process as claimed in claim 38, wherein the aqueous medium in step (a) further comprises a protein denaturant.

45. The process as claimed in claim 39, wherein the protein denaturant is selected from the group consisting of urea, guanidine hydrochloride, and thiocyanate.

46. The process as claimed in claim 44, wherein the native transglutaminase concentration is from 40 mg/ml and the protein denaturant concentration is from 4 to 10 M.

47. The process as claimed in claim 38, wherein the acidic aqueous medium in step (a) is of a pH from 2 to 7.

48. The process as claimed in claim 38, wherein the acidic aqueous medium in step (a) is of a pH from 3 to 5.

49. The process as claimed in claim 38, wherein the acidic aqueous medium in step (a) is of a pH from 3.5 to 4.5.

50. The process as claimed in claim 38, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 5-fold.

51. The process as claimed in claim 38, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted at least 10-fold.

52. The process as claimed in claim 38, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted from 50-fold to 400-fold.

53. The process as claimed in claim 38, wherein said incubation is performed at not more than 15°C.

54. The process as claimed in claim 38, wherein said incubation is performed from 3 to 10°C.

55. The process as claimed in claim 38, wherein preceding step (b), the acidic aqueous medium of said denatured transglutaminase is diluted to a concentration of not more than 10 mg/ml.

56. The process as claimed in claim 38, wherein said neutral pH is from 5.8 to 8.5.

57. The process as claimed in claim 38, wherein said neutral pH is from 6 to 7.

58. The process as claimed in claim 38, wherein in step (b), the aqueous medium further comprises an accelerator for forming a higher-order native-state transglutaminase structure having enzymatic activity.

59. The process as claimed in claim 58, wherein the accelerator is selected from the

group consisting of an inorganic salt, an organic salt, an amino acid salt, a polyol, an organic solvent, and a surfactant.

60. The process as claimed in claim 59, wherein the inorganic salt accelerator is selected from the group consisting of calcium chloride and strontium chloride.

61. The process as claimed in claim 59, wherein the inorganic salt accelerator concentration is from 0.01 to 10 mM.

62. The process as claimed in claim 59, wherein the organic salt accelerator is selected from the group consisting of sodium acetate and sodium propionate.

63. The process as claimed in claim 59, wherein the organic salt accelerator concentration is from 0.1 to 2 M.

64. The process as claimed in claim 59, wherein the amino acid salt accelerator is arginine hydrochloride.

65. The process as claimed in claim 59, wherein the amino acid salt accelerator concentration is from 0.1 to 2 M.

66. The process as claimed in claim 59, wherein the polyol accelerator is polyethylene glycol.

67. The process as claimed in claim 59, wherein the polyol accelerator concentration is from 1 to 10%.

68. The process as claimed in claim 59, wherein the organic solvent accelerator is selected from the group consisting of DMSO and DMF.

69. The process as claimed in claim 59, wherein the organic solvent accelerator concentration is from 10 to 40%.

70. The process as claimed in claim 59, wherein the surfactant is CHAPS.

71. The process as claimed in claim 59, wherein the surfactant concentration is from 1 to 50 mM.

72. The process as claimed in claim 38, further comprising:

(c) a step for separating inactive enzyme(s) as aggregate(s) by centrifugation.

73. An isolated transglutaminase obtained by the process of claim 38, which has an intermediate structure having a molecular ellipticity which is 30 to 70% of that of a native-state transglutaminase in a CD spectrum of a near ultraviolet region.

74. The process as claimed in claim 38, wherein step (b) further comprises incubating the transglutaminase for more than 1.5 hours subsequent to adjusting the pH to a neutral region to allow for complete refolding of the native state.

75. A transglutaminase comprising the following properties (a) to (d):

(a) specific activity of 15 to 25 U/mg provided through measurement of transglutaminase activity by the hydroxamate method;

(b) a molecular ellipticity which is 30 to 70% of that of the native state in a CD spectrum of a near ultraviolet region;

(c) a molecular weight of 36,000 to 40,000 as measured by SDS-polyacrylamide gel electrophoresis; and

(d) lower mobility than that of a native state in native-polyacrylamide gel electrophoresis with a His-Mes buffer system of pH 6.1.